STIC-ILL 370,577 Q11.N26 NUC

From: Arthur, Lisa
Sent: Monday, November 05, 2001 2:22 PM
To: STIC-ILL
Subject: reference for 08/484,786

Please provide a copy of the following:

1. Conner et al. (1983) PNAS 80: 278-282.

2. Rollini et al. PNAS (1985 Nov) 82(12): 7197-7201.

3.Gorski et al. IMMUNOGENTICS (1987) 25(6):379-402.

4.. de Preval et al. IMMUNOGEENTICS (1987) 26(4-5): 249-257.

5. Irle et al. J. EXPERIMENTAL MEDICINE (1988 Mar 1) 167(3): 853-872.

6. Andersson et al. IMMUNOGENETICS (1988) 28 (1): 1-5.

Thanks,
Lisa Arthur
CM1 12E13
1655
308-3988

.5 of wk
11/7 R.C

Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 278–282, January 1983
Medical Sciences

Detection of sickle cell β^S-globin allele by hybridization with synthetic oligonucleotides

(sickle cell anemia/prenatal diagnosis/genetic disease)

BRENDA J. CONNER*, ANTONIO A. REYES†, CHRISTOPHE MORIN‡, KEIICHI ITAKURA†, R. L. TEPLITZ*, AND R. BRUCE WALLACE†

*Division of Cytogenetics and Cytology, City of Hope Medical Center, and †Division of Biology, Molecular Genetics Section, City of Hope Research Institute, Duarte, California, 91010; and ‡Laboratorie de Chimie, Institut Pasteur, Paris, France

Communicated by Norman H. Horowitz, September 23, 1982

ABSTRACT **Two 19-base-long oligonucleotides were synthesized, one complementary to the normal human β-globin gene (β^A) and one complementary to the sickle cell β-globin gene (β^S). The nonadecanucleotides were radioactively labeled and used as probes in DNA hybridization. Under appropriate hybridization conditions, these probes can be used to distinguish the β^A gene from the β^S allele. The DNA from individuals homozygous for the normal β-globin gene ($\beta^A\beta^A$) only hybridized with the β^A specific probe; the DNA from those homozygous for the sickle cell β-globin gene ($\beta^S\beta^S$) only hybridized with the β^S specific probe. The DNA from heterozygous individuals ($\beta^A\beta^S$) hybridized with both probes. This allele-specific hybridization behavior of oligonucleotides provides a general method for diagnosis of any genetic disease which involves a point mutation in the DNA sequence of a single-copy gene.**

Synthetic oligodeoxyribonucleotides have been shown to hybridize specifically to complementary DNA sequences (1–3). Under appropriate hybridization conditions, only perfectly base-paired oligonucleotide·DNA duplexes will form; duplexes containing a single mismatched base pair will not be stable. This high degree of hybridization specificity has led to the development of a general method for using synthetic oligonucleotides as specific probes to identify cloned DNAs coding for proteins of interest. Recently, this technique has been applied to the successful isolation of a human β_2-microglobulin cDNA clone (4) as well as a murine transplantation antigen cDNA clone (5).

Because a mutation in a single base in the DNA sequence of a gene would affect the hybridization behavior of an oligonucleotide complementary to the region of the mutation (2), oligonucleotide hybridization has the potential to provide a method of detecting single-base changes within genomic DNA. Point mutations are the cause of a substantial number of human genetic diseases (6). Synthetic oligonucleotides, therefore, could be used as specific probes for determination of genotype and aid in diagnosis of genetic disease, even prenatally.

We chose the β-globin gene as a model system to test the applicability of using synthetic oligonucleotides to detect a point mutation within a single copy gene. The β-globin gene is a member of the single-copy β-globin-like gene family which includes the ε-, $^G\gamma$-, $^A\gamma$-, δ-, and β-globin genes arranged 5′ to 3′ in order of expression during development (7). Recently, these genes have been cloned, restriction maps have been defined, and much of the DNA sequence has been determined for the coding and immediate flanking regions (8–11). Sickle cell anemia, a human genetic disease found predominantly in the Black population, is the result of a single base pair (bp) change (adenine to thymine) in the β-globin gene, corresponding to the sixth amino acid residue (changing glutamic acid to valine) in the β-globin protein (12, 13). The sickle cell disorders follow a single-gene Mendelian mode of inheritance. Instead of the normal β-globin genotype ($\beta^A\beta^A$), individuals with sickle cell trait have one normal β-globin gene and one sickle cell allele ($\beta^A\beta^S$); those with sickle cell disease have two sickle cell alleles ($\beta^S\beta^S$) and no β^A gene.

In this report, we show that nonadecanucleotides complementary to the β-globin gene (β^A) or to the sickle cell allele (β^S) in the region of the sickle cell point mutation can: (*i*) distinguish β^A from β^S as well as from other members of the β-globin-like gene family, (*ii*) specifically detect the single-copy β-globin gene in human genomic DNA, and (*iii*) allow the unambiguous determination of β-globin genotype of individuals, confirming the hematological diagnosis of sickle cell trait or sickle cell disease. Applied to prenatal diagnosis of sickle cell disorders, this procedure offers several advantages over methods now available. More significantly, these techniques may be applied generally for diagnosis of genetic diseases that involve a specific change—such as a base substitution, insertion, or deletion—in the DNA sequence of a single gene.

MATERIALS AND METHODS

Chemical Synthesis of Oligodeoxyribonucleotides of Unique Sequence. Nonadecanucleotides (Table 1) were synthesized on a solid support by the modified triester approach as described (14).

^{32}P-Labeling of Synthetic Oligonucleotides. Synthetic oligonucleotides were labeled with adenosine 5′-[γ-^{32}P]triphosphates (ICN, crude preparation, >7,000 Ci/mmol; 1 Ci = 3.7 × 10^{10} Bq) by a kinase reaction (1). Separation of labeled oligonucleotide from unlabeled nonadecanucleotide and reaction products by homochromatography (15) yielded oligonucleotide probes with specific activities of approximately 2 × 10^9 cpm/μg.

Source of Recombinant DNA and Transformants. λ-HβG1 and λ-HγC5 and pBR322–HβPst clones were a generous gift of T. Maniatis. The pBR322–HβPst plasmid contained a 4.4-kilobase (kb) *Pst* I fragment of β^A subcloned in the *Pst* I site of pBR322 (16). DNA was isolated as described (3, 17). Recom-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: bp, base pair(s); kb, kilobase(s); β^A, normal β-globin gene; β^S, sickle cell β-globin gene; $\beta^A\beta^A$, normal β-globin genotype; $\beta^A\beta^S$, sickle cell trait genotype; $\beta^S\beta^S$, sickle cell disease genotype; λ-HβG1, recombinant bacteriophage constructed with DNA insert of human δ-globin and β-globin genes; λ-HγG5, recombinant bacteriophage constructed with DNA insert of human $^G\gamma$- and $^A\gamma$-globin genes; pBR322-HβPst, recombinant plasmid constructed with insert of human β-globin gene.

Table 1. DNA sequence of synthetic oligonucleotide probes for normal β-globin gene (β^A) and sickle cell β-globin allele (β^S)

Gene	Probe	DNA sequence
β^A	Hβ19A	$^{5'}$CT CCT GAG GAG AAG TCT GC$^{3'}$
	Hβ19A′	$^{3'}$GA GGA CTC CTC TTC AGA CG$^{5'}$
β^S	Hβ19S	$^{5'}$CT CCT GTG GAG AAG TCT GC$^{3'}$
	Hβ19S′	$^{3'}$GA GGA CAC CTC TTC AGA CG$^{5'}$

binant DNA was handled in accordance with the National Institutes of Health guidelines.

Isolation of Human DNA. Peripheral blood samples (10–20 ml) were collected from scientific personnel (normal for β-globin) or from patients with sickle cell trait ($\beta^A\beta^S$) or sickle cell disease ($\beta^S\beta^S$); all gave informed consent. DNA was isolated from leukocytes as described (18) with some modifications. Instead of dialysis, the DNA was extracted three times with phenol/chloroform, 3:1 (vol/vol), and treated with RNase (19). The average yield was 50 μg of DNA per ml of whole blood.

Restriction Endonuclease Digestion of DNA. λ-HβG1 DNA and pBR322–Hβ*Pst* DNA were digested at 1 enzyme unit/μg of DNA at 37°C for 30 min. Human DNA (10 μg) was digested at 5 enzyme units/μg of DNA at 37°C for 4 hr; additional endonuclease was then added at 5 units/μg of DNA and digestion was continued for 12 hr or overnight. The restriction endonucleases used were *Bam*HI, *Eco*RI, and *Hpa* I (Boehringer Mannheim). *Bam*HI and *Hpa* I digestions were carried out in 7 mM NaCl/7 mM Tris·HCl, pH 7.5/7 mM dithiothreitol/7 mM magnesium acetate. The buffer for *Eco*RI was 100 mM Tris, pH 7.2/5 mM magnesium acetate/100 mM NaCl/0.02% Nonidet P-40. When double digestions were required, the amounts of enzyme and times of digestion were as described above, except the endonuclease requiring low-salt buffer was used first. The buffer was then adjusted to higher salt conditions for digestion with *Eco*RI. Digestions were terminated by heating the sample at 65°C for 3 min.

Electrophoresis and Hybridization of DNA. Digested DNAs were loaded into individual wells of a vertical (1 or 3 mm thick) 1.0–1.2% agarose gel (SeaKem), electrophoresed at 1 V/cm at 50 V for 750 V hr in a Tris borate electrophoresis buffer, stained with ethidium bromide (Calbiochem) at 0.25 μg/ml for 30 min, and photographed over UV light (3, 20). The DNA was denatured *in situ* and transferred to nitrocellulose paper (21). Alternatively, the gel itself was dried for direct hybridization (22). After ethidium bromide staining, the gel was denatured with 0.5 M NaOH and 0.15 M NaCl at room temperature for 30 min and neutralized in 0.5 M Tris, pH 8.0/0.15 M NaCl at 4°C for 30 min. The gel was then dried under vacuum onto Whatman 3MM paper with a Hoefer gel dryer. The dry gel was rinsed in 0.15 M NaCl to remove backing paper. Nitrocellulose paper or the dried gel was sealed in plastic (Dazey Seal-A-Meal), hybridized, and washed as specified in the figure legends. The nitrocellulose or gel was blotted dry with Whatman 3MM paper, wrapped in Saran Wrap, and autoradiographed between two Quanta III intensifier screens (DuPont) at −80°C for 4 hr or 3–5 days.

RESULTS

Rationale of Specificity of Oligonucleotide Hybridization. The DNA sequences of the synthetic oligonucleotides used in this study are given in Table 1. The position and length of the sequence of the oligonucleotides were based on several criteria. (*i*) The oligonucleotide was designed to be 19 nucleotides long in order that this sequence would have a high probability of recognizing a unique sequence (23). Hβ19A and Hβ19A′ were specific for normal β-globin DNA (β^A); Hβ19S and Hβ19S′ were specific for sickle cell β-globin DNA (β^S). (*ii*) The sickle cell mutation was positioned near the center of the sequence to maximize thermal instability of mismatch hybridization. (*iii*) The sequences synthesized were not complementary to the ε-, ^{G}γ-, ^{A}γ-, or δ-globin genes in the region of probe complementarity (Table 2).

The Hβ19A′ oligonucleotide (or Hβ19A) should form a perfect hybrid with the β^A DNA (Table 3). If the sickle cell point mutation (β^S) is present, there would be one mismatched nucleotide at the site of the point mutation in the oligonucleotide·DNA duplex with Hβ19A′ (T/T mismatch) or Hβ19A (A/A mismatch) probe. Conversely, Hβ19S′ (or Hβ19S) oligonucleotide probe should hybridize perfectly with β^S DNA. However, one mismatched nucleotide would be present in a duplex of β^A DNA and Hβ19S′ (A/A mismatch) or Hβ19S (T/T mismatch) oligonucleotide.

Table 2. Amino acid sequence and DNA sequence of human β-globin-like genes in the region of oligonucleotide hybridization

Gene	5′ 1				5					10	3′	Mismatches, no. Hβ19A	Hβ19S
ε	...Met	Val	His	Phe	Thr	Ala	Glu	Glu	Lys	Ala	Ala	Val ...		
	...ATG	GTG	CAT	TTT	ACT	*GCT*	GAG	GAG	AAG	*GCT*	GCC	GTC...	2	3
^{G}γ	...Met	Gly	His	Phe	Thr	Glu	Gly	Asp	Lys	Ala	Thr	Ile ...		
^{A}γ	...ATG	GTG	CAT	TTC	AC*A*	*GAG*	GAG	GAC	AAG	*GCT*	ACT	ATC...	7	8
δ	...Met	Val	His	Leu	Thr	Pro	Glu	Glu	Lys	Thr	Ala	Val ...		
	...ATG	GTG	CAT	CTG	ACT	CCT	GAG	GAG	AAG	ACT	GCT	GTC...	1	2
β^A	...Met	Val	His	Leu	Thr	Pro	Glu	Glu	Lys	Ser	Ala	Val ...		
	...ATG	GTG	CAC	CTG	ACT	CCT	GAG	GAG	AAG	TCT	GCC	GTT...	0	1
β^S	...Met	Val	His	Leu	Thr	Pro	Val	Glu	Lys	Ser	Ala	Val ...		
	...ATG	GTG	CAC	CTG	ACT	CCT	*GTG*	GAG	AAG	TCT	CCC	GTT...	1	0

Italic nucleotides represent position of base-pair differences relative to the β^A. The connecting line represents the position of oligonucleotide hybridization.

Table 3. Type of base-pair mismatch in duplexes of synthetic oligonucleotide probe and DNA of β^A and of β^S

Probe	β-Globin allele	
	β^A	β^S
Hβ19A	Perfect match	A/A mismatch
Hβ19A′	Perfect match	T/T mismatch
Hβ19S	T/T mismatch	Perfect match
Hβ19S′	A/A mismatch	Perfect match

Selectivity of Oligonucleotide Hybridization to Temperature. The effect of temperature on oligonucleotide hybridization to human β-globin DNA is shown in Fig. 1. pBR322–HβPst DNA was digested with *Bam*HI and subjected to electrophoresis in agarose gels in eight identical lanes; the DNA was denatured *in situ* and transferred to nitrocellulose by the standard Southern procedure (21). For each duplicate, one lane was hybridized with 5′-^{32}P-labeled Hβ19A (perfect match) and the other with 5′-^{32}P-labeled Hβ19S (T/T mismatch) (Table 1). The temperature of the hybridization and the washes that followed were then varied as indicated. With hybridization at 45°C and wash at 0°C, hybridization to the 1.8-kb *Bam*HI restriction fragment containing the 5′ end of the β-globin gene was evident with the Hβ19A (perfect match) and, to a lesser extent, with the Hβ19S (T/T mismatch) probe. Based on the length and DNA sequence of the oligonucleotide, it was calculated that 55°C would be a temperature at which the nonadecanucleotide·DNA complex would be stable only if base pairing were perfect (3). Hybridization of the gel at 45°C followed by a wash at 55°C removed hybridization with the Hβ19S probe (T/T mismatch), allowing stable hybridization only with the Hβ19A probe (perfect match). At a hybridization temperature of 55°C, only the Hβ19A (perfect match) probe hybridized to the 1.8-kb restriction fragment. Under these stringent conditions, only perfectly matched oligonucleotide·DNA duplexes were stable. Hybridization at the higher temperature increased specificity but resulted in a slightly decreased signal (compare 45/55 with 55/55 in Fig. 1).

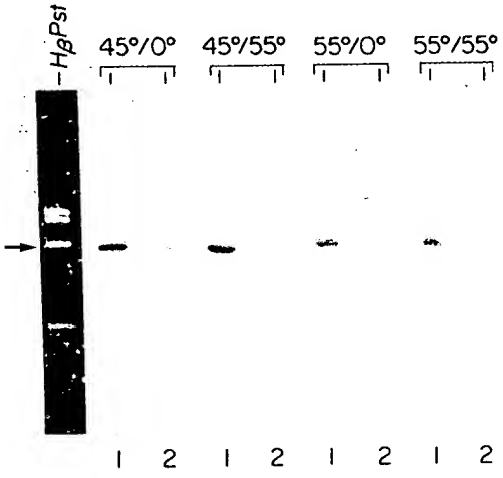

FIG. 1. Effect of temperature on the hybridization of oligonucleotides to globin DNA. Individual lanes of pBR322–HβPst DNA (1 ng) digested with *Bam*HI were hybridized for 16 hr in hybridization buffer [5× Denhardts (1× modified Denhardts is 0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% NaDodSO$_4$/0.02% Ficoll) containing 10% dextran sulfate (Pharmacia or Sigma), 6× NET (1× NET is 0.15 M NaCl/0.03 M Tris·HCl, pH 8.0/1 mM EDTA), and 0.5% Nonidet P-40]. The buffer also contained (10^6 cpm/ml) of a labeled oligonucleotide probe [5′-^{32}P]Hβ19A (perfect match) (lanes 1) or [5′-^{32}P]Hβ19S (T/T mismatch) (lanes 2). After hybridization at 45°C or 55°C as indicated, each nitrocellulose paper strip was washed with three changes (15 min each) of 0.9 M NaCl/0.09 M sodium citrate at 0°C. Half of the filters were then washed for 1 min at 55°C, as indicated (the numbers at the top of the figure indicate hybridization temperature first and wash temperature second). The ethidium bromide-stained gel is shown at the left. Arrow, position of the 5′ end of the β-globin gene.

Oligonucleotide Probes Can Differentiate β-Globin Gene from Other β-Globin-Like Genes. The stringent hybridization conditions established above were used to determine if the oligonucleotide probes could also distinguish the β-globin gene sequence from those of the β-globin-like genes. The recombinant bacteriophage λ-HβG1 contains both the δ- and β-globin genes; λ-HγG5 contains the human ^{G}γ- and ^{A}γ-globin genes (17). There is one base-pair difference in the region of Hβ19A hybridization between δ- and β-globin genes, and seven nucleotide changes in this region between ^{G}γ- or ^{A}γ- and β-globin (Table 2). These two DNAs were digested with *Eco*RI and hybridized with 5′-^{32}P-labeled Hβ19A (Fig. 2). At 45°C the labeled probe hybridized to the fragment containing δ-globin (T/T mismatch) as well as to the fragment containing the β-globin gene in λ-HβG1 DNA (perfect match). At 55°C, only hybridization to the fragment containing the β-globin gene was evident. The λ-HγG5 DNA, with seven noncomplementary bases in the region of hybridization, did not hybridize to the probe at either temperature.

Detection of β-Globin Gene in Human Genomic DNA. Because the sequence of the β-globin gene has been determined and extensive restriction maps have been established (11), it is possible to predict the fragment sizes expected to contain the 5′ region of the β-globin gene if total human genomic DNA were digested with various restriction endonucleases. Fig. 3A shows the results for digestion of λ-HβG1 DNA and human genomic DNA normal for the β-globin gene ($\beta^A\beta^A$) with *Bam*HI or double digestion with either *Bam*HI/*Eco*RI or *Hpa* I/*Eco*RI. From the known restriction map of the β-globin gene (11), the sizes of restriction fragments predicted to contain the 5′ end of the β-globin gene are: 1.8 kb, *Bam*HI; 1.8 kb, *Bam*HI/*Eco*RI; 2.2 kb, *Hpa* I/*Eco*RI (Fig. 3*B*). λ HβG1 was similarly digested and included on the gel as a marker. In the *Bam*HI

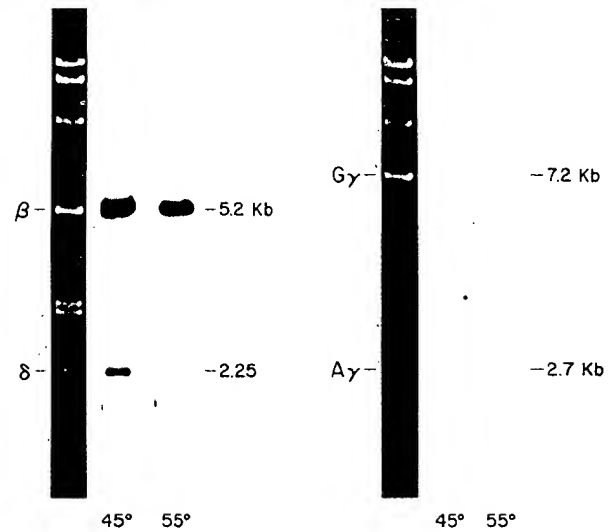

FIG. 2. Hybridization of ^{32}P-labeled Hβ19A to ^{G}γ-, ^{A}γ-, δ-, and β-globin genes. Duplicate samples of λ-HβG1 DNA (*Left*) or λ-HγG5 (*Right*) (0.25 μg) were digested with *Eco*RI and hybridized with 5′-^{32}P-labeled Hβ19A probe at either 45°C or 55°C for 18 hr (as in Fig. 1). The positions of the *Eco*RI restriction fragments containing the 5′ end of each globin gene are noted to the left of the ethidium bromide-stained gels.

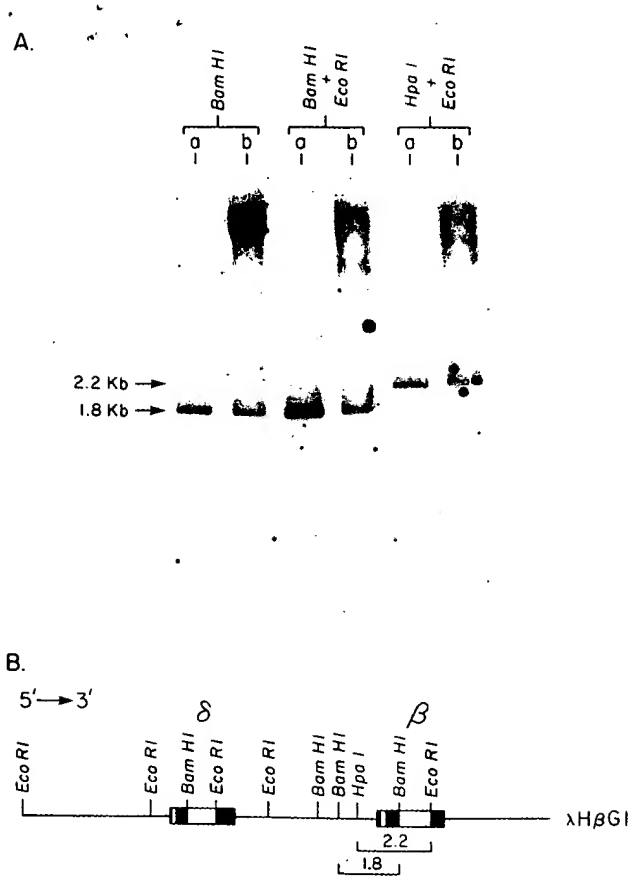

FIG. 3. Hybridization of ^{32}P-labeled Hβ19A′ to human genomic DNA digested with different restriction endonucleases. (*A*) λ-HβG1 DNA (lanes a) or 10 μg of human genomic DNA (lanes b) ($\beta^A\beta^A$) was subjected to single or double digestion as indicated. After electrophoresis, the agarose gel was dried, hybridized with [5′-^{32}P]Hβ19A′ at 10^7 cpm/ml for 2 hr at 55°C, washed at 0°C (Fig. 1), and then given a 1-min wash in 0.9 M NaCl/0.09 M sodium citrate at 55°C. λ-HβG1 was loaded at 150 pg as a control for intensity of hybridization of a single copy gene, except that in the *Bam*HI/*Eco*RI double digestion 300 pg of DNA was loaded. (*B*) Localization of selected restriction enzyme sites for *Eco*RI, *Bam*HI, and *Hpa* I (10). The expected sizes of the *Bam*HI or *Bam*HI/*Eco*RI fragment (1.8 kb) and the *Hpa* I/*Eco*RI fragment (2.2 kb) are indicated.

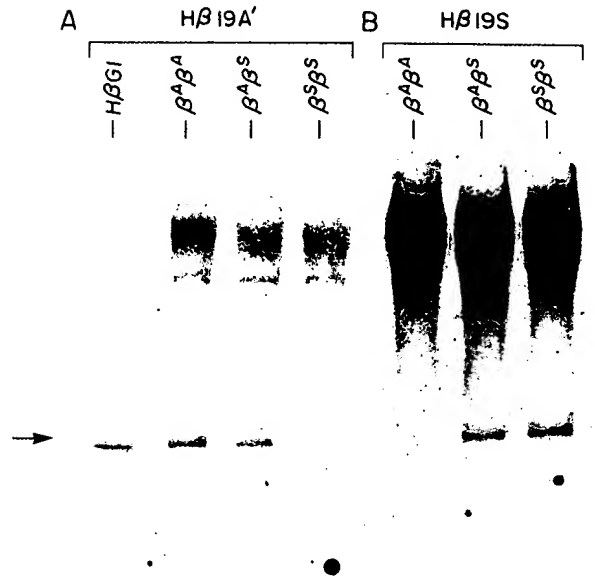

FIG. 4. Hybridization of [^{32}P]nonadecanucleotide (Hβ19A′ and Hβ19S) to human genomic DNA. λ-HβG1 DNA (150 pg) and duplicates of genomic DNAs (10 μg) $\beta^A\beta^A$, $\beta^A\beta^S$, and $\beta^S\beta^S$, were digested with *Bam*HI and electrophoresed. The gel was dried and half was hybridized with [5′-^{32}P]Hβ19A′ (*A*); the other half was hybridized with [5′-^{32}P]Hβ19S (*B*) at 10^7 cpm/ml for 2 hr at 55°C. The gels were washed at 0°C and twice, for 1 min each, in 0.9 M NaCl/0.9 M sodium citrate at 55°C. The gel pieces were realigned and autoradiographed. The 1.8-kb fragment containing the 5′ end of the β-globin gene is indicated by the arrow.

and the *Hpa* I/*Eco*RI lanes, the amount of λ-HβG1 DNA loaded was equivalent to a single-copy gene in 10 μg of human DNA (5×10^{-6} pmol). In each digestion, the hybridizing band in the genomic digests comigrated with the restriction fragments containing the 5′ end of the β-globin gene in the λ-HβG1 marker DNA (Fig. 3*A*). In addition, the level of hybridization of the hybridizing band in the genomic digests was as expected for a single-copy gene, based on the hybridization obtained for the λ-HβG1 DNA.

Determination of Gene Dosage of β^A and β^A Allele in Human Genomic DNA. Genomic DNAs from patients previously diagnosed as normal for β-globin ($\beta^A\beta^A$) or having sickle cell trait ($\beta^A\beta^S$) or sickle cell disease ($\beta^S\beta^S$) were digested with *Bam*HI endonuclease and subjected to electrophoresis in duplicate. For one half of the gel, the genomic DNAs with an appropriate marker for the 5′-end of β^A gene (λ-HβG1 digested with *Bam*HI) were hybridized with Hβ19A′ probe (β^A, perfect match; β^S, T/T mismatch) (Fig. 4*A*). The duplicate lanes were hybridized with Hβ19S probe (β^A, T/T mismatch; β^S perfect match) (Fig. 4*B*). In Fig. 4*A*, the 1.8-kb *Bam*HI restriction fragment hybridized with Hβ19A′ for normal ($\beta^A\beta^A$) and sickle cell trait ($\beta^A\beta^S$) genomic DNA. The level of hybridization to the 5′ end of the globin gene fragment for normal DNA was noticeably greater than that for sickle cell trait DNA. Very little hybridization was seen with ($\beta^S\beta^S$) DNA with the Hβ19A′ probe (T/T mismatch). When the Hβ19S probe was hybridized in the duplicate lanes, the probe hybridized to the 1.8-kb fragment in the $\beta^S\beta^S$ and $\beta^A\beta^S$ DNAs but not to the $\beta^A\beta^A$ DNA (T/T mismatch) (Fig. 4*B*). Again, the level of hybridization with the Hβ19S probe was greater with $\beta^S\beta^S$ DNA than with $\beta^A\beta^S$ DNA.

DISCUSSION

We have demonstrated in this report that synthetic oligonucleotides recognizing a specific sequence of DNA can detect a single-copy gene in human genomic DNA. The nonadecanucleotide probes can differentiate the β-globin gene from other members of the β-globin-like gene family and can distinguish the normal β-globin gene (β^A) from the β^S allele, a difference of a single nucleotide change.

In this study, 1 bp mismatch out of 19 bp decreased the thermal stability of the oligonucleotide·DNA duplex. Hybridization and wash conditions were established that would allow discrimination of β^A DNA from DNAs containing a single base change such as δ-globin and β^S genes, as well as other genes in the β-globin-like gene family. The type of nucleotide mismatch—e.g., A/A, T/T, etc.—could be expected to have an effect on the stability of the complex. However, our results showed that the hybridization patterns of nonadecanucleotide·DNA duplexes with a T/T bp mismatch [Hβ19A′ with β^S DNA, Hβ19S

with β^A DNA (Fig. 4)] were similar to those obtained with an A/A bp mismatch (Hβ19A with β^S DNA, Hβ19S′ with β^A DNA; data not shown).

The ability of synthetic oligonucleotides to detect a point mutation within this sequence was tested by hybridization with genomic DNA from individuals normal for β-globin ($\beta^A\beta^A$) or having the sickle cell allele (β^S) in the heterozygous or homozygous state. The oligonucleotide probes Hβ19A′ and Hβ19S hybridized to their respective genes in an allele-specific manner (Fig. 4). Significantly, the intensity of labeling at the hybridized β-globin gene fragment for each probe was proportional to gene dosage. The genotype of the β-globin gene could be determined from the hybridization pattern, confirming diagnoses made previously by hemoglobin typing (S. Rahbar, personal communication) (24). The reason for the hybridization in the upper region of the gel for genomic DNA samples (Figs. 3 and 4) is not known. However, it is not likely that this binding represents partial or incompletely digested fragments because of the experimental conditions used.

The technique of hybridization with synthetic oligonucleotides yields results equivalent to analysis with *Mst* II endonuclease (25–27). Both procedures offer direct analysis, and both utilize small amounts of DNA. A limitation of the *Mst* II analysis is that *Mst* II will not distinguish the β^C from the β^S allele because the β^C mutation (GAG to AAG, glycine to lysine, sixth codon, β-globin) occurs at the N position (N = any) of the *Mst* II recognition sequence (C-C-T-N-A-G-G). Oligonucleotide probes, on the other hand, are specific for the $\beta^A$and β^S alleles.

The most significant advantage this technique offers is that it has the potential to be applied to the diagnosis of any genetic disease in which a specific change in DNA sequence is involved, particularly in the case of base substitution but also for insertion or deletion not analyzable by any other methods. The sequence of the oligonucleotide can be designed precisely according to need. This eliminates dependence on restriction enzyme recognition site alteration, which has a low probability of occurrence for any given point mutation (23). Therefore, this technique can be applied not only to the diagnosis of the sickle cell allele (β^S) but also to detection of the β^C mutation and the single base changes recently reported for β-thalassemia (see ref. 28 for review), α-thalassemia (29), and α_1-antitrypsin deficiency disorders (30). These point mutations, which do not affect any known restriction endonuclease recognition site, could be detected readily by an appropriate oligonucleotide probe as described herein.

We thank T. Maniatis for the β-globin clones, S. Rahbar for the gift of blood samples, and C. C. Impraim for assistance in the final stages of the work. R.B.W. and K.I. are members of the Cancer Research Center (CA16434) at the City of Hope Research Institute. The stay of C.M. was made possible in part by a grant from the North Atlantic Treaty Organization. This work was supported by National Institutes of Health Grant HL 29516 (R.B.W.) and Health and Welfare Agency, Genetic Disease Section, State of California Grant 81-77521-A1 (B.J.C. and R.L.T.).

1. Wallace, R. B., Shaffer, J., Murphy, R. F., Bonner, J., Hirose, T. & Itakura, K. (1979) *Nucleic Acids Res.* **6**, 3543–3557.
2. Wallace, R. B., Johnson, M. J., Hirose, T., Miyake, T., Kawashima, E. H. & Itakura, K. (1981) *Nucleic Acids Res.* **9**, 879–894.
3. Wallace, R. B., Schöld, M., Johnson, M. J., Dembek, P. & Itakura, K. (1981) *Nucleic Acids Res.* **9**, 3647–3656.
4. Suggs, S. V., Wallace, R. B., Hirose, T., Kawashima, E. H. & Itakura, K. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6613–6617.
5. Reyes, A. A., Schöld, M., Itakura, K. & Wallace, R. B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3270–3274.
6. Neel, J. V. (1978) *Can. J. Genet. Cytol.* **20**, 295–306.
7. Maniatis, T., Fritsch, E. F., Lauer, J. & Lawn, R. M. (1980) *Annu. Rev. Genet.* **14**, 145–178.
8. Baralle, F. E., Shoulders, C. C. & Proudfoot, N. J. (1980) *Cell* **21**, 621–626.
9. Slightom, J. L., Blechl, A. E. & Smithies, O. (1980) *Cell* **21**, 627–638.
10. Spritz, R. A., DeRiel, J. K., Forget, B. G. & Weissman, S. M. (1980) *Cell* **21**, 647–651.
11. Lawn, R. M., Efstradiatis, A., O'Connell, C. & Maniatis, T. (1981) *Cell* **21**, 652–654.
12. Pauling, L., Itano, H. A., Singer, S. J. & Wells, I. C. (1949) *Science* **110**, 543–548.
13. Marotta, C. A., Wilson, J. T., Forget, B. G. & Weissman, S. M. (1977) *J. Biol. Chem.* **252**, 5040–5053.
14. Dembek, P., Miyoshi, K. & Itakura, K. (1981) *J. Am. Chem. Soc.* **103**, 706–708.
15. Jay, E., Bambara, R., Padmanabhan, R. & Wu, R. (1974) *Nucleic Acids Res.* **1**, 331–353.
16. Fritsch, E. F., Lawn, R. M. & Maniatis, T. (1980) *Cell* **19**, 959–972.
17. Lawn, R. M., Fritsch, E. F., Parker, R. C., Black, G. & Maniatis, T. (1978) *Cell* **15**, 1157–1174.
18. Kan, Y. W., Dozy, A. M., Trecardin, R. & Todd. D. (1977) *N. Engl. J. Med.* **297**, 1081–1084.
19. Goossens, M. & Kan, Y. W. (1981) *Methods Enzymol.* **76**, 805–817.
20. Peacock, A. C. & Dingman, C. W. (1968) *Biochemistry* **7**, 668–674.
21. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
22. Shinnick, T. M., Lund, E., Smithies, O. & Blattner, F. R. (1975) *Nucleic Acids Res.* **2**, 1911–1929.
23. Nei, M. & Li, W.-H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5269–5273.
24. Steinberg, M. H. & Adams, J. G., III (1978) *South. Med. J.* **71**, 413–416.
25. Chang, J. C. & Kan, Y. W. (1982) *N. Engl. J. Med.* **307**, 30–32.
26. Orkin, S. H., Little, P. F. R., Kazazian, H. H. & Boehm, C. D. (1982) *N. Engl. J. Med.* **307**, 32–36.
27. Wilson, J. T., Milner, P. F., Summer, M. E., Nallaseth, F. S., Fadel, H. E., Reindollar, R. H., McDonough, P. G. & Wilson, L. B. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 362S–3631.
28. Orkin, S. H., Kazazian, H. H., Jr., Antonarakis, S. E., Goff, S. C., Boehm, C. D., Sexton, J. P., Weber, P. G. & Giardina, P. J. V. (1982) *Nature (London)* **296**, 627–631.
29. Goossens, M., Lee, K. Y., Liebhaber, S. A. & Kan, Y. W. (1982) *Nature (London)* **296**, 864–865.
30. Kurachi, K., Chandra, T., Degen, S. J. F., White, T. T., Marchioro, T. L., Woo, S. L. C. & Davie, E. W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6826–6830.